

US-PAT-NO: 6462258

DOCUMENT-IDENTIFIER: US 6462258 B1

TITLE: Plant expression constructs

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US Patent No. - PN (1):
6462258

Detailed Description Text - DETX (140):

Cotton yield is correlated with the number of squares set during the first four to five weeks of squaring. The retention of these squares to mature bolls and their contribution to the harvest of the cotton lint is a key component of yield. When determining the efficacy of transgene constructs for conferring herbicide tolerance in cotton, the amount of boll retention is a measure of efficacy and is a desirable trait. Transgenic cotton plants containing promoters of the present invention (Table 7) were assayed in greenhouse conditions for boll retention. The promoters directed expression of the aroA:CP4 coding sequence for glyphosate tolerant phenotype.

The plants were transformed by an Agrobacterium-mediated method or by a particle gun method. The particle gun constructs contained an additional GUS containing expression cassette useful for histochemical localization of .beta.-glucuronidase activity from the promoters of the present invention. Transgenic plants were regenerated on glyphosate containing media and plants rooted on a rooting media. The rooted plantlets were potted in soil and transferred to a growth chamber for a hardening off period. The seed from these

plant lines were collected and planted. Fifteen plants from each line were sprayed with glyphosate at 48 ounces/acre at the 4 leaf stage. At least 8 surviving plants from each line were sprayed again at the 8 leaf stage with glyphosate at 48 ounces/acre. At maturity, the number of first position bolls for the first five bolls was counted. Those lines that had 3 or more of the first position bolls retained after the glyphosate spray (plant map.gstoreq.3) were advanced for further study. Table 7 illustrates the data produced from this study. The number of lines mapped indicates the number of lines surviving the first glyphosate spray application. The commercial standard is Line 1445 (pMON17136) that contains the P-FMV promoter driving expression of the CTP2-aroA:CP4 gene/E9 3', this line retains less than 1 of the 5 first bolls. The constructs, pCGN8099, pCGN9153, pCGN8088, pCGN8068 provided sufficient reproductive glyphosate tolerance in cotton such that 14-35% of the lines tested from these constructs were advanced for further agronomic trials.

Detailed Description Text - DETX (142):

Cotton yield is correlated with the number of squares set during the first four to five weeks of squaring. The retention of these squares to mature bolls and their contribution to the harvest of the cotton lint is a key component of yield. When determining the efficacy of transgene constructs for conferring herbicide tolerance in cotton, the amount of boll retention is a measure of efficacy and is a desirable trait. Transgenic cotton plants containing promoters of the present invention were assayed in field conditions at two locations for boll retention. The transgenic cotton lines 502-254-2

(pCGN8068), 701-178-2 (pCGN8068), 53-2 (pCGN8088), 178-1 (pCGN9153), and 60-1 (pCGN9153) were compared to 1445 (glyphosate tolerance line) and PM1218BR (Paymaster 1218 parent) that contain the construct pMON17136 (P-FMV/CTP2-aroA:CP4/E93'), a wild type non-transgenic line, Coker 130 was included. The field design is a randomized complete block design consisting of 2 rows.times.20-30 feet.times.3 replications. Glyphosate is applied as Roundup Ultra.TM. formulation at rates of 1.12 lb ai/A=48 oz product and 1.5 lb ai/A=64 oz product at the 8 leaf stage of cotton plant development. All of the cotton plots are managed aggressively for weed and insect pest control, as well as other agronomic inputs such as planting time, fertilization, irrigation, PGR usage and defoliation. The percent boll retention is determined by mapping the location of each of the retained bolls by random selection of ten plants from the middle of the two center rows (five from each row) of each plot to map. The first mapping should be done 4 weeks after first flower (mid-season map), a second mapping should be done at harvest. The data collected includes the number of first position bolls on the bottom five flowering nodes that are counted as an indication of the reproductive tolerance of the transgenic cotton lines to glyphosate. Table 8 illustrates the advantage that promoters of the present invention have conferred to transgenic cotton plants for boll retention. This enhanced reproductive tolerance has resulted in increased lint yield (Table 9) and increased seed yield (Table 10) as well.

Detailed Description Paragraph Table - DETL (8):

TABLE 7	Greenhouse cotton boll retention study	#
lines	Construct	
Promoters Mapped	Plant Map	.gtoreq. 3 % .gtoreq.3 pCGN8099

FMV:EF1a + 104 36
 34.6% e35S:Act8 pCGN9153 EF1.alpha. + FMV 36 12 33.3%
 pCGN9165 EF1.alpha.
 +35S/GUS 3 1 33.3% pCGN9152 EF1a 7 0 0.0% pCGN8088 Act8 +
 FMV 43 6 14.0%
 pCGN8086 Act8 7 0 0.0% pCGN8068 Act2 + FMV 37 7 18.9%
 pCGN8067 Act2 37 0 0.0%
 pCGN8084 Act2 + FMV + 5 0 0.0% 35S/GUS pCGN8085 Act2 +
 FMV/GUS 1 0 0.0%
 pCGN9164 Act11 +35S/GUS 21 1 4.8% pMON45325 Act11 + FMV 43
 0 0.0% pCGN8096
 FMV:Act11 + 14 0 0.0% e35S:Act2 pCGN9154 FMV:Act11 + 16 1
 6.3% e355:Act2
 Line 1445 FMV <1.0

Detailed Description Paragraph Table - DETL (9):

TABLE 8 Boll retention at mid-season plant map of
 bottom 5 first position
 bolls Location 2 Location 1 Un- 48 64 Untreated 48 oz/A
 64 oz/A treated oz/A
 oz/A (17136) 1445 68 67 53 81 63 62 (8068) 502-254-2 87
 72 64 77 80 69
 (8068) 701-178-2 85 77 60 84 86 76 (8088) 53-2 89 81 80 79
 76 73 (9153) 178-1
 77 83 73 85 71 79 (9153) 60-1 80 89 81 77 82 87 PM1218BR
 92 56 63

Detailed Description Paragraph Table - DETL (10):

TABLE 9 Lint Yield (lbs/Acre) and percent yield
 (Location 1) Cultivar
 Untreated 48 oz/A 48 oz/A % 64 oz/A 64 oz/A %
 8068-502-254-2-4 1103 960 87.0%
 858 77.8% 8068-701-178-2-2 1326 1219 91.9% 1177 88.8%
 9153-60-1-1 1177 1206
 102.5% 1171 99.5% 9153-178-1-1 1112 769 69.2% 750 67.4%
 8088-53-2-11 1283
 1071 83.5% 1097 85.5% 1445 1018 563 55.3% 490 48.1% C130
 1200 0 0.0% 0 0.0%
 PM 1218 BR 1092 826 75.6% 713 65.3%

Detailed Description Paragraph Table - DETL (11):

TABLE 10 Seed Cotton Yield (lbs/Acre) and percent
 yield (Location 1)
 Cultivar Untreated 48 oz/A 48 oz/A % 64 oz/A 64 oz/A %
 8068-502-254-2-4 3357